Research Plan Proposal

Comparative evaluation of the effectiveness of Pentoxifylline and Clomiphene citrate with Ayurvedic Shilajit and Ashwgandha in Cadmium induced male Reproductive toxicity

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Introduction

Reproductive hazards from metals exposure in males are one of the fastest growing areas of concern in toxicology today. During the past 50 years, tens of thousands of metals, chemicals have been released into the general environment (Carbone *et al,* 2007).

The rapid industrialization and overgrowing urbanization, the effects of metals on male reproductive system have become major health concern globally (Turgnt *et al,* 2003). Over exposure to different heavy metals causes irreversible toxic insult to male reproductive system. The toxicity may be expressed as alteration to the female or male reproductive organs, the related endocrine system or pregnancy out comes.

Mammalian male reproductive function can be affected through a direct effect on the testis, resulting in decreased or altered sperm production, through impairment of the accessory sex gland secretion and directly through the neuro endocrine system , causing hormonal imbalance (Chandra *et al,* 2012).

Recent evidences indicate that the human male reproductive capacity has deteriorated considerably during the past five decades. In industrialized countries, a substantial number of couples seek *in vitro* fertilization or intracytoplasmic sperm injection because of poor semen quality (Nordberg *et al* ,2005).

Cadmium is ubiquitous environmental pollutant. Cadmium is considered as one of the most toxic heavy metals and a potent industrial pollutant that adversely affects various organs in human or animals (De Sousa *et al* , 2011).

Cadmium is widely distributed in the environment due to extensive uses of cadmium and cadmium based products such as nickel cadmium batteries, television, phosphors stabilizer or polyvinyl chloride etc, moreover owing to its brilliant orange color. It is extensively used as pigment paints, plasters and plastic (Singh *et al,* 2012).

Exposure to cadmium can occur from food, water, smoking cigarette and at place by inhalation of fumes and dust (Ashraf, 2012). Its concentration in food samples vary widely but the highest average is detected in mollusk, kidneys, vegetables (Oqungbe and Hawal, 2003).

The estimates of cadmium dietary intake derived from the WHO, regional diets based on food balance sheets ranged from 0.35 to 9.63 microgram/BW per day (Bellinger, 2004).The possible mechanism of cadmium induced toxicity is oxidative stress generation of reactive oxygen species resulting in oxidative deterioration of lipids, proteins and DNA and initiating various pathological conditions in human and animals (Cypers *et al* ,2010).Cadmium exposure is strongly associated with reproductive toxicity in both animals and human population culminating in infertility and cancer of the reproductive tissues (Rekha *et al* ,2011).

Cadmium causes alteration in the serum levels of androgens and other reproductive hormones as well as histo-pathological changes to reproductive organs (Massanyi *et al* ,2007, Aprioku *et al* ,2009). Cadmium exposure can adversely affect male fertility and results in severe impairment of testicular functions including germ cell death and inhibition of testicular steroidogenesis (Yang *et al* , 2003, Gunnarsson, 2007).

Oxidative stress is a condition associated with an increased rate of cellular damage induced by oxygen and oxygen –derived oxidants commonly called as reactive oxygen species (Zikic *et al*, 1998).

Cellular damage in the semen is the result of an improved balance between reactive oxygen species generation and scavenging activities. The scavenging potential in gonad and seminal fluid is normally maintained by adequate levels of antioxidants super dismutase, catalase, glutathione (Shi *et al,* 1999).

Long term exposure to cadmium increased lipid peroxidation and caused inhibition of super dismutase activity indicating oxidative damage in liver ,kidney and testes (Patra *et al*,1999).The pathogenesis of testicular damage following cadmium exposure is generally described to oxidative damage (Yiin *et al*,1999).Although the testis expresses several antioxidants enzymes such as superoxide dismutase ,catalase and glutathione peroxidase to counteract the oxidative stress ,their levels are greatly diminished upon cadmium exposure. (Sen *et al*, 2004).

Recently, Azoospermic persons were found to have higher serum and seminal plasma cadmium level compared with oligospermic ones (Oluyemi *et al,* 2006). Also positive relationship was found between cadmium exposure and astheno-zoospermia in a Rat model (Benoff *et al*, 2008).

Allopathic Drugs

Allopathy is derived from the "Greek" word "*allos"*(others or different) + *"pathos"* (disease or suffering) which means other than the disease.

Allopathy is basically a drug oriented methodology and relies on three things hypothesis, experimentation and the result of the experiments .The effectiveness of allopathic medicines during an emergency is the fundamental reason why it is adopted. There are multiple medicines that may be used to treat reproductive toxicity.

Pentoxifylline

At present, methylxanthine derivaties such as pentoxifylline are used increasingly in reproductive medicine, mainly for their enhancing effect on sperm motility. Pentoxifylline and other methylxanthines are known to influence sperm metabolism and motility.

Pentofylline is reported not only to stimulate sperm motility (Aparicio *et al*, 1980) but also improve fertilization rates in humans *in vitro* fertilization (Yovich *et al*,1990). Pentoxifylline is a 3,5- nucleotidase phosphodiesterase inhibitor that enhances sperm motility by increasing intracellular c-AMP ,subsequently provokes both cellular glycolysis generation and endogenous adenosine tri phosphate ATP production that lead to increased sperm movements consequently (Suji and Sivakami,2003).

It is considered to be a sperm movement enhancer (Tesarik *et al* 1992a), hyper activation agent, inhibition of reactive oxygen species formation (Yovich, 1993) and acrosome reaction –improvement agent (Tesarik *et al,* 1992b).

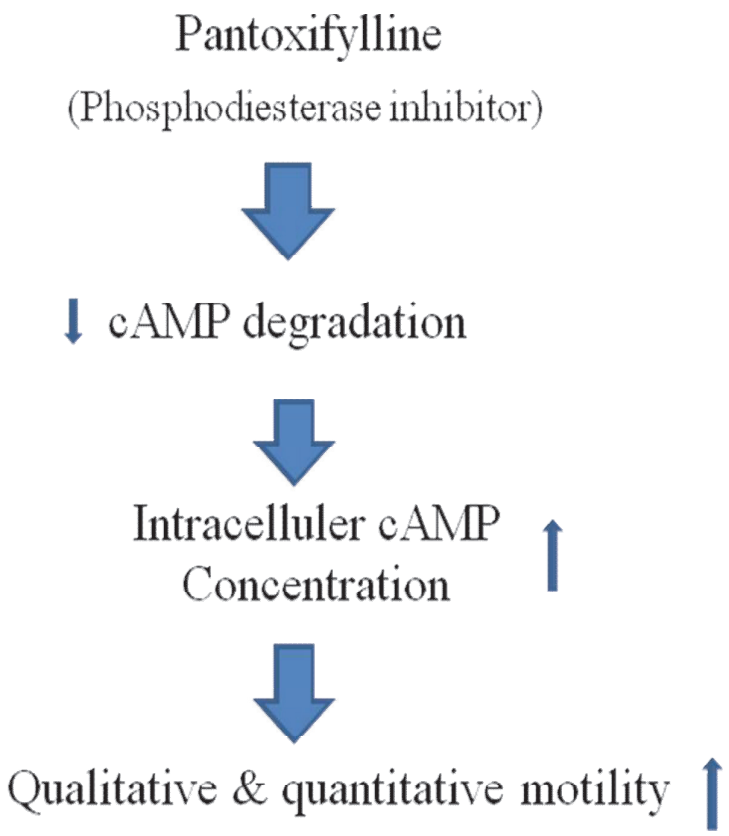


Fig. Mechanism of Pantoxifylline

Clomiphene citrate

It is an orally active non steroidal agent distantly related to diethylstilbestrol. It stimulate pituitary gonadotropin release by excluding estradiol from hypothalamic receptor sites (Abdul *et al*,1996).This interaction neutralizes the normal negative feedback control of estrogen and results in enhanced secretion of LH-RH, FSH-RH and gonadotropins .Testosterone is produced by leydig cells in response to LH secretion .The concentration of testosterone in the tubular environment is believed to maintain the gametogenic function of testis (Elliason *et al*,1975).

Clomiphene citrate stimulates adrenal androgen biosynthesis selectively (Fuse *et al* 1993). It also increases seminal transferring levels as well as stimulates the sertoli cells, which help in the better supply of iron to the developing germ cells resulting in better production of spermatocytes (Skinner *et al* 1980).

- Mechanism of action-

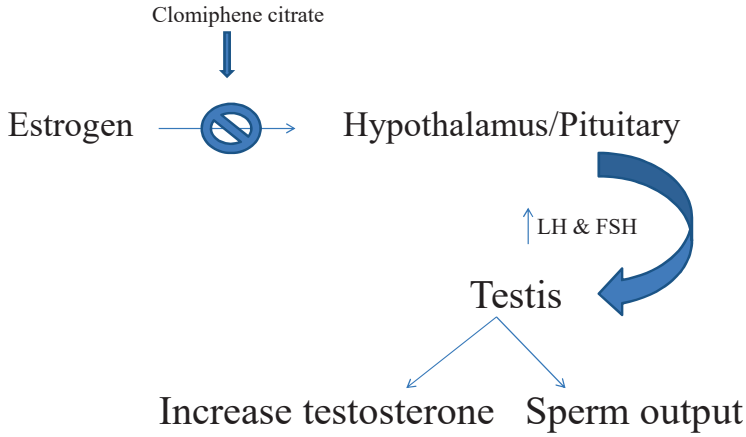


Fig. Mechanism of Clomiphene citrate

Ayurvedic Drugs

Ayurveda is a Sanskrit word is formed two words "*Ayus*" meaning life and "*Veda*" so the term Ayurveda resembles the knowledge of life. Ayurveda is also called as the science of natural healing because it derives its medicines purely from nature. It has been proved that Ayurveda is Mother of all medication.

The demand of herbal drugs is increasing day by day in developed as well as developing countries because they are safer and well tolerated as compared to those of allopathic drugs. Medicinal plants should be subjected to animal and human studies to determine their effectiveness (Singh *et al ,2012).*

Shilajit (*Asphaltum*)

Asphaltum is a yellowish brown to blackish gummy secretion obtained from the different mountain ranges of the world .It exudates from the layers of rocks specially in the Himalayas and Hindukush mountains of subcontinent.

It is composed of at least 85 minerals which are in the ionic form, includes triterpens and aromatic carboxylic acid. Other ingredients identified in *Asphaltum* are moisture, gums, aluminium, calcium, potassium, nitrogen, silica, resin, vegetable matter,magnesium,sulfur,iron,chloride,phosphorous,iodine,glycosides,tannic acid ,benzoic acid and a number of vitamins and enzymes (Khanna *et al*,2008).

It is having a natural quality to increase the sensitivity of nerves in the penis .This improvement in sensitization in turn will increase the testosterone production, which results in boosting the sex drive and desire (Wilson *et al,* 2011).

Ashwgandha (*Withania somenifera)*

India has a rich history of using plants for medicinal purpose. The Indian system of medicine viz Ayurveda , Unani and homeopathic system predominantly use plant –based raw materials for most of their preparation and formulations.

Ashwgandha is a medicinal plant extensively used in Ayurveda as home remedy for various disease (Mathur *et al* ,2006).Ashwgandha also known as *Withania somenifera* ,winter cherry ,Indian ginseng has been an important traditional herbal medicine for over 3000 years (Mishra *et al* 2005).The roots of this plant include some alkaloids and vitanolids (Sangwan *et al*,2008).

Ashwgandha induces some changes in hypophysical gonadotropines accompanied by an increase in sperm in male rats and folliculogenesis in immature female rats (Al-Qaraweit *et al*, 2011). Ashwgandha is often called "Indian ginseng" due to its effects (Mishra *et al*,2012).

Ashwgandha inhibits lipid peroxidation and reduces oxidative stress in men, mice and rats (Ahmad *et al*, 2009). Ashwgandha has been found to counteract the formation of reactive oxygen species in infertile male (Manish and Jayalakshmi, 2006).

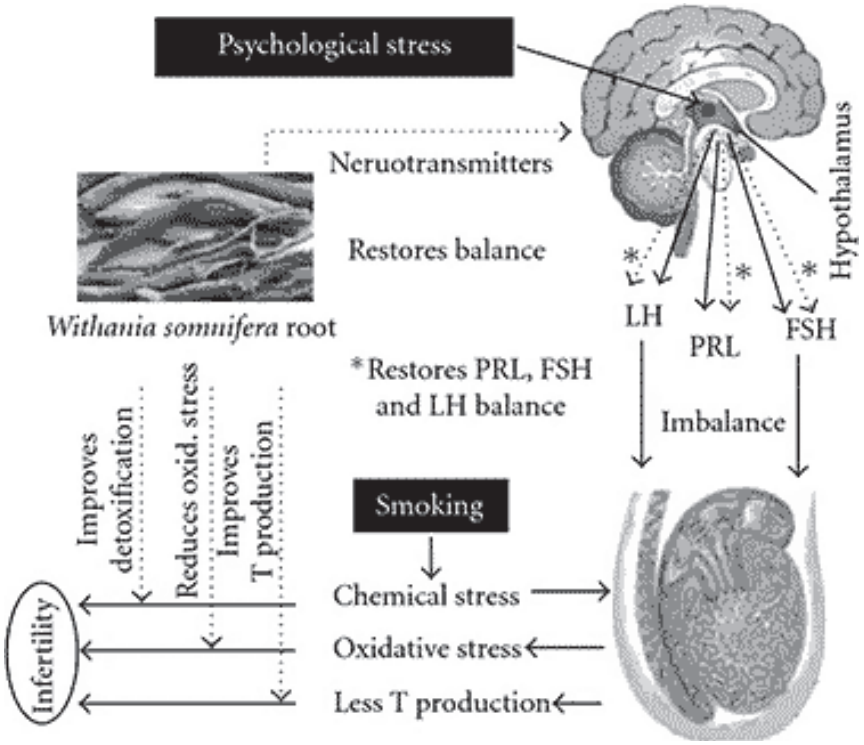


Fig. Mechanism of Ashwagandha

Review of Literature

In 2000 Susan *et al* reported that lead and cadmium both have the ability to undergo an acrosomal reaction decrease. Yan li *et al* in 2010 reported that Cadmium exposure markedly reduced the weights of testis, epidymidus and prostrate and seminal vesicle in adult mice.

Yasmin *et al in 2011* suggested that Cadmium has deleterious effects on spermatogenesis and Cadmium induces oxidative damage on testis cell.

Alaee *et al* in 2014 examined that elevated level of Cadmium induced toxic effects on total male reproductive system. Tribow *et al,* 2014 examined Cadmium induced oxidative stress in ovarian cells. Rania *et al* in 2014 suggested that Cadmium chloride administration in low doses adversely affect the reproductive functions in rats.

Yovich in 1993 published a review on Pentoxifylline action and application reproduction and concluded that Pentoxifylline has proven effects on sperm motility, increasing the proportion of hyperactivated spermatozoa

In 2000,Terriou *et al* concluded Pentoxifylline enhance sperm motility in many in vitro fertilization program. Behnaz *et al* in 2011 reported oral administration of pentoxifylline enhances the sperm progressive motility and acrosomal reaction.

Administration of L-carnitine and Pentoxifylline enhanced sperm motility (Elham *et al* 2013). Pentoxifylline is a potentially beneficial agent to reduce testicular damage and improves sperm parameters in diabetic mice by decreasing the ratio of apoptosis (Feyli *et al* 2016).

Simantini *et al* in 2007 suggested that Clomiphene citrate exerts its effects on spermatogenesis by raising the endogenous semen FSH, LH and testosterone levels to initiate and maintain gametogenesis.

Shail *et al* in 2014 concluded that Clomiphene citrate could be beneficial to protect granulosa cell as well as oocyte apoptosis and inhibit deterioration on oocyte quality. Rohulu *et al*, 2015 observed Clomiphine citrate stimulated spermatogenesis in low dose but higher depressed spermatogenesis.

Stohs in 2013 concluded that Shilajit exhibits antioxidant ,anti-inflammatory, adaptogenic, immunomodulatory, anti- dislipidemic properties and also observed that Shilajit enhances spermatogenesis in rats. In 2016 Muhammad *et al* evaluated that Shilajit may lead to increase number of sperms, motility, mass activity and circumference of the testes.

Ranjit *et al*, 2012 illustrated effect of Endosulfan on biochemical, hormonal and sub cellular anomalies of spermatozoa of mice and restoration through root extract of *Withania somenifera*. In 2013, Gupta *et al* concluded that the aqueous extract of Shilajit can be useful in the treatment of certain forms of sexual inadequacies such as premature ejaculation and oligospermia.

Objective-

1. To evaluate Cadmium induced male reproductive toxicity.
2. Analysis of effectiveness of –

   a) Pentoxifylline and Clomiphene citrate.

   b) Ayurvedic Shilajit and Ashwgandha.

   c) Pentoxifylline and Clomiphene citrate to Ayurvedic Shilajit and Ashwgandha after Cadmium induced male toxicity.
3. Evaluation of spermatogenesis and steroidogenesis after Cadmium induced male Reproductive toxicity.

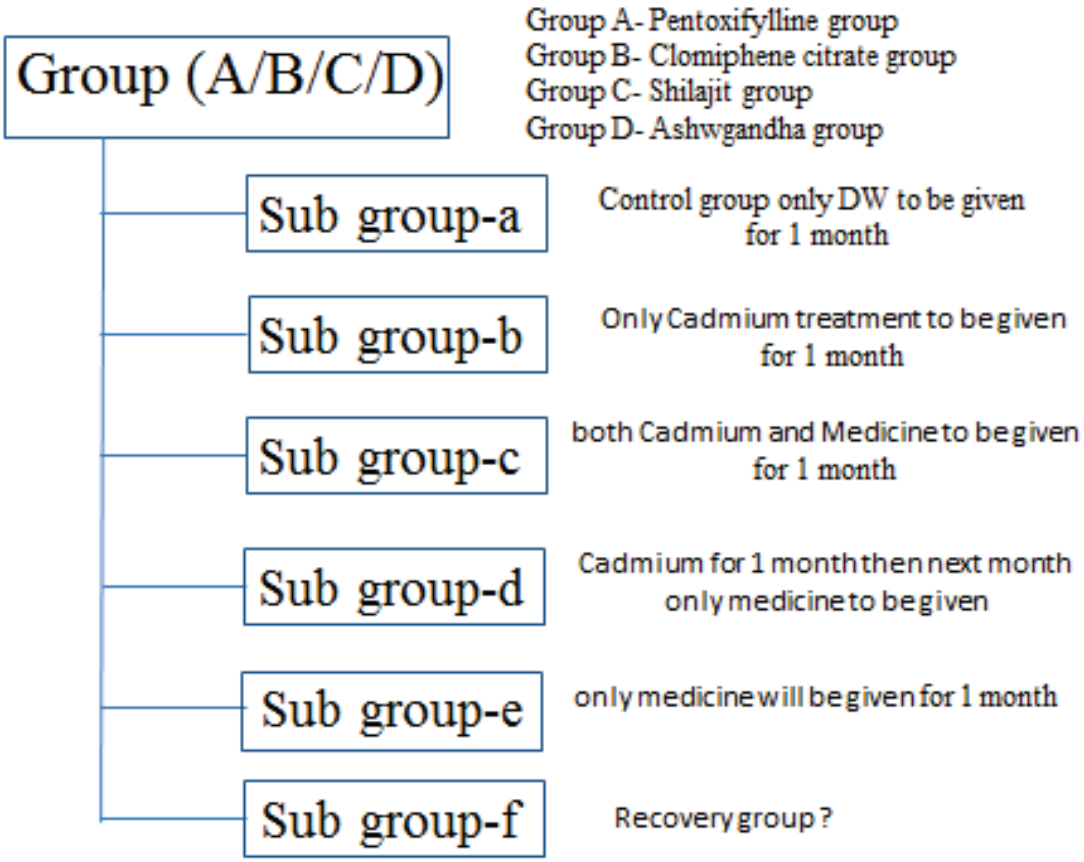
Plan of Work

Experimental Animal

- Animal to be use for this study will be male albino Swiss mice which will be procured from the animal house of ICG campus. Weighing between 25 to 30 mg animals will be use for this study.
- Mice will be keep in the cages with wood shavings under standard condition.
- Mice will be feed on standard diet and water .

Experimental protocol-

Mice will be divided into four medicinal group viz. A, B, C and D. Further divided into six sub groups. Each contains 5 Animal.


Group (A/B/C/D)
Group A- Pentoxifylline group
Group B- Clomiphene citrate group
Group C- Shilajit group
Group D- Ashwgandha group
Sub group-a
Control group only DW to be given for 1 month
Sub group-b
Only Cadmium treatment to be given for 1 month
Sub group-c
both Cadmium and Medicine to be given for 1 month
Sub group-d
Cadmium for 1 month then next month only medicine to be given
Sub group-e
only medicine will be given for 1 month
Sub group-f
Recovery group ?

Mode of Treatment - Orally

Duration of Experiment - 1 month

Autopsy will be done at 7, 15 and 30 days, for subgroup- d autopsy interval will be 45 and 60 days also.

At the termination of experimental (week) animals will be sacrificed and the sample will be collected for further analysis or assessment.

Parameters- which will done by following methods

A .Sperm Analysis-

- Sperm Motility and Density (Prasad *et al*, 1972).
- Sperm deformalities **(**Bruce *et al*, 1974).
- Sperm Viability **(**Bjorndahl *et al*, 2003).
- Weight of Testis, Epididymis, Prostate.

B. Serum hormone level

1. Testosterone
2. LH and FSH

C. Histopathology of testis

D. Enymatic test-

1. Lipid Peroxidation (Ohkawa *et al*,1979).
2. Glutathione
3. Sodium Dismutase (Morkund and Morkund,1974).
4. Ascorbic acid (Roe and Kuether,1943).

E. Tissue (Testis) Biochemistry

1. Total Protein (Lowry *et al*,1951)).
2. Glycogen
3. Sialic acid (Warren, 1957).
4. Total Cholesterol (Zlatkis *et al,*1953).
5. Acid Phosphatase and Alkaline Phosphatase (Kind and King,1954).

F. Markers of Steroidogenesis-

1. 17-beta Hydroxy steroid dehydrogenase (Talalay,1962).
2. 3-beta Hydroxy steroid dehydrogenase (Talalay,1962).

G. Immunohistochemistry-

1. Aromatase Assay

Statistical Analysis-

Comparison of the values will be done between drugs (Pentoxifylline and Clomiphene citrate) and Ayurvedic (Shilajit and Ashwgandha) treated group of mice. Statistical evaluation of data will be done following Student "t" test and ANOVA.

Significance of work-

To prove effectiveness and efficacy between drugs (Pentoxifylline and Clomiphene citrate) and Ayurvedic (Shilajit and Ashwgandha) by scientific experimentation in the treatment of Cadmium induced Male reproductive toxicity.

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